

## Effects of Thyroxin on Amino Acid Incorporation into Protein

**Abstract.** The effect of thyroxin on the *in vitro* incorporation of DL-leucine-1- $C^{14}$  into the protein of rat liver homogenates has been investigated. Both thyroxin pretreatment *in vivo* and thyroxin *in vitro* at a concentration of  $1 \times 10^{-5}M$  were found to increase the rate of amino acid incorporation. The increased activity following the thyroxin pretreatment *in vivo* was found to be localized in the mitochondrial fraction. It is suggested that the acceleration of metabolic rate characteristic of thyroxin action may be secondary to the stimulation of energy-requiring reactions such as protein synthesis.

Recent concepts of the mechanism of action of thyroxin have emphasized its uncoupling effect on oxidative phosphorylation (1, 2). This effect, however, is observed only with relatively high concentrations of thyroxin, occurs equally well with both the D- and L-forms (1), and, except for changes in oxidative metabolism, explains few of the physiological effects of the thyroid hormone. Many clinical features of thyroid disease suggest a major, if not primary, role of the thyroid hormone in protein metabolism. In immature animals it is involved in growth; in adults it causes pronounced changes in nitrogen metabolism. Furthermore, in the adult brain and testis—organs in which the quantities of protein and lipid turned over per unit time are apparently negligible compared with turnover of carbohydrate, as evidenced by a respiratory quotient of approximately 1 (3)—the characteristic acceleration of metabolic rate that is observed in almost all other tissues is absent in hyperthyroidism (4).

Previous observations (5) have indicated that thyroxin pretreatment *in vivo* stimulates amino acid uptake into the protein of rat liver slices. To investigate further the apparent relationship between thyroid function and protein synthesis, studies were undertaken to determine the effects of *in vivo* and *in vitro* thyroxin administration on the *in vitro* incorporation of DL-leucine-1- $C^{14}$  into the proteins of rat liver homogenates. Livers from 90- to 150-g fasting, male Sprague-Dawley rats were homogenized by means of glass homogenizers in 5 ml of 0.25M sucrose solution per gram of tissue. Homogenization was performed at 0° to 2°C, and tissue fractions were maintained at that temperature through all subsequent operations until final incubation. Intact cells, nuclei, and cell debris were removed by centrifugation at 700g for 10 minutes. The supernatant fluid was spun at 54,000g for 60 minutes in a

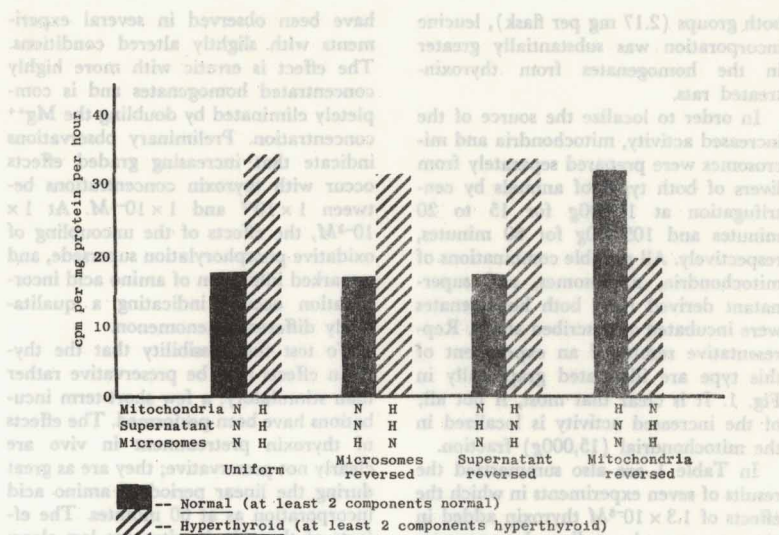


Fig. 1. Localization of increased amino acid incorporating activity in fractions of liver homogenates from rats pretreated with thyroxin. Results are representative of seven such experiments.

Spinco model L ultracentrifuge. The sediment, containing both mitochondrial and microsomal fractions, was suspended in appropriate amounts of 0.25M sucrose and supernatant fluid to yield a suspension containing particulate fractions and supernatant fluid equivalent to approximately 200 mg and 30 mg of liver, respectively, per 0.45 ml, the quantity of homogenate added to each of the experimental flasks.

In eight experiments rats were paired for age and weight; one received almost daily intraperitoneal injections of 100  $\mu$ g of sodium thyroxin in 1 ml of 0.01N NaOH; the other received equivalent amounts of the NaOH solution alone. After at least six doses in 7 days, homogenates were prepared simultaneously from both animals as described above, and DL-leucine-1- $C^{14}$  incorporation activity in both was measured in parallel flasks in a single combined experiment. Flask contents and incubation procedure are described in the title of Table 1. The reaction was terminated with 12-percent trichloroacetic acid, and the precipitated protein was purified and plated on filter paper by a modification of the method of Siekevitz (6). Sample weights were determined from difference in planchet weights before and after plating. Radioactivity was measured with a thin-window Geiger-Mueller counter; total counts collected were sufficient to yield a 3-percent coefficient of variation. Counting rates were corrected for background, self-absorption, and zero time controls. The results are summarized in Table 1. Although protein nitrogen concentrations, as determined by the micro-Kjeldahl technique, were identical in

Table 1. Effects of thyroxin on DL-leucine-1- $C^{14}$  incorporation into protein of rat-liver homogenates. To each flask (25-ml Erlenmeyer) were added 5  $\mu$ mole of adenosine-5'-monophosphate, 20  $\mu$ mole of potassium phosphate (pH 7.4), 5  $\mu$ mole of  $MgCl_2$ , 50  $\mu$ mole of potassium  $\alpha$ -ketoglutarate, 0.8  $\mu$ mole of DL-leucine-1- $C^{14}$  (specific activity, 5.33  $\mu$ Ci/ $\mu$ mole), and 0.45 ml of the appropriate homogenate prepared in 0.25M sucrose, as described in the text. In *in vitro* studies, 0.022  $\mu$ mole of sodium thyroxin contained in 0.1 ml of 0.01N NaOH was added to the experimental flasks; all other flasks received equivalent amounts of the NaOH solution alone. The reaction mixture was brought to a final volume of 1.7 ml with 0.25M sucrose. Incubation in air was carried out with shaking in a water bath at 37°C for 1 hour. Zero time controls were included in all experiments.

Item	Activity (count/min mg of protein per hr)	
	Mean	Standard error
<i>Thyroxin pretreatment in vivo</i> (8 rat pairs)		
Normal rat	29.0	$\pm 1.9$
Hyperthyroid rat	42.3	$\pm 3.0$
Difference	13.3*	$\pm 3.2$
Effect (%)	+46	
<i>Treatment with <math>1.3 \times 10^{-5}M</math> thyroxin</i> <i>in vitro</i> (7 experiments)		
Control	26.9	$\pm 1.8$
Thyroxin-treated	31.9	$\pm 2.3$
Difference	5.0*	$\pm 1.4$
Effect (%)	+19	

\* Denotes statistical significance;  $p < .02$  (determined by method of paired comparison).



